CRYSTALLIZATION AND CRYSTAL DATA OF THAUMATIN I, A SWEET-TASTING PROTEIN FROM *THAUMATOCOCCUS DANIELLII* BENTH

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1. Introduction

Thaumatin I is one of the sweet-tasting proteins from the fruit of *Thaumatococcus danielii* benth, a plant growing in tropical Africa [1]. Its mol. wt. is 21 000 as calculated from ultra-centrifugal data [1]. The molecule contains 193 amino acids [2]; polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate [3] indicated that the protein is a single polypeptide chain with alanine as the N-terminal amino acid [1]. Psychophysical experiments [4] revealed that the sweetness of the protein is dependent on both the temperature and pH, and completely disappears above a certain temperature which varies with pH. Circular dichroism measurements confirmed these results [5]. The impression of sweet taste is also destroyed after cleavage of the disulphide bonds in the protein. All this points to the tertiary structure being of importance for the taste activity. To obtain more insight into this relationship, knowledge of the tertiary structure, which can be obtained only by X-ray crystallography, is necessary.

We here describe the crystallization of Thaumatin I and present physical characteristics and diffraction data of the crystals obtained and needed for further elucidation of the tertiary structure after formation of heavy atom derivatives.

2. Materials and methods

Thaumatin I, obtained as described in [1] was crystallized by the free interface diffusion technique [6]. We used glass tubes with an inner diameter of 3 mm and a length of 50 mm at a temperature of 20°C, and 0.15 ml of a 1% or a 2% solution of Thaumatin I in bidistilled water and 0.2 ml 80% satured ammonium sulphate solution in bidistilled water (516 g/l).

The Thaumatin I crystals, in the presence of the mother liquor, were mounted in glass capillaries. To produce the diffraction photographs we used a Nonius precession camera; the unit cell dimensions and crystallographic symmetry were determined with a Philips PW 1100 automatic diffractometer. Both the camera and the diffractometer were equipped with a copper fine-focus tube.

3. Results

Two different crystal modifications of Thaumatin I were obtained. The 1% solution of Thaumatin I produced regularly formed bipyramids with linear dimensions of 0.4–0.6 mm. The unit cell, determined with the diffractometer in an automated way using the 'peak hunt' program developed by Hornstra of the Philips Research Laboratories, is tetragonal with a = b = 58.5 Å and C = 151.8 Å; V = 519500 Å.^{3}

The reflection conditions h00, h = 2n and 00*l*, *l* = 4n, prove the space group to be either $P4_1 2_1 2$ or (its enantiomorph) $P4_3 2_1 2$. Fig. 1. shows a 20° precession photograph of the Ok*l* section through reciprocal space. This photograph was taken from the same crystal as that used for the unit cell determination experiments. In the direction of the tetragonal axis, reflections can be observed down to spacings of 2.4 Å; however, in the direction of the long reciprocal axis, reflections already disappear below 3.2 Å.

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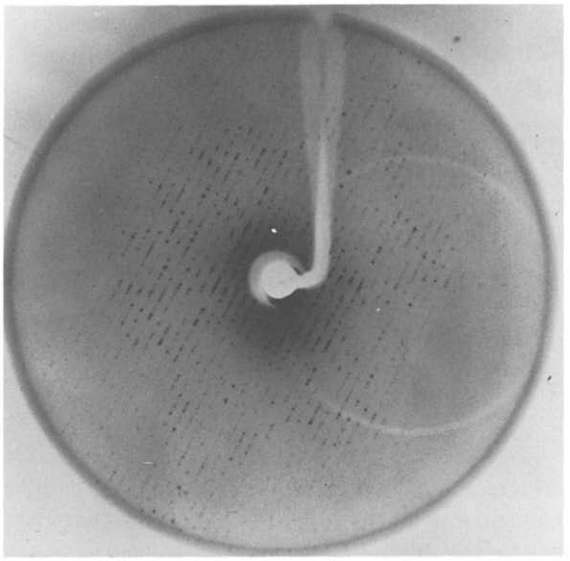

Fig.1. A 20° precession photograph of the 0k*l* reflections of the tetragonal modification of Thaumatin I.

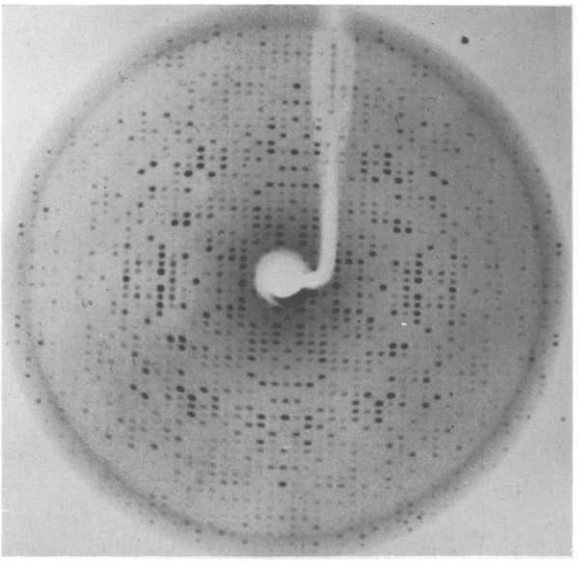

Fig.2. A 20° precession photograph of the hk0 reflections of the orthorhombic modification of Thaumatin I.

A second form of Thaumatin I crystals was obtained with the 2% solution. A lath-shaped crystal of dimensions 1.0 × 0.4 × 0.4 mm^3 was taken for the precession photograph reproduced in fig.2 and for the determination of the unit cell data with the diffractometer. The crystal appeared to be orthorhombic with a = 73.04, b = 52.89 and c = 52.32 Å; V = 202116 $Å^3$. From the reflection conditions h00, h = 2n, 0k0, k = 2n and 00*l*, *l* = 2n the space group is found to be $P2_12_12_1$. The 20° precession photograph (fig.2) shows reflections to at least 2.3 Å resolution.

Acknowledgement

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